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A Comparison of the Treatment of Cyanide Poisoning in the Cynomolgus Monkey with Sodium Nitrite or 4-Dimethylaminophenol (4-DMAP), with and without Sodium Thiosulfate

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February 1994

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13. ABSTRACT *(Maximum 200 words)*
Two methemoglobin generating compounds, sodium nitrite (iv) or 4-dimethylamino-phenol (4-DMAP (im), with and without sodium thiosulfate (iv), were compared as post-treatment therapy in anesthetized monkeys poisoning with cyanide. Arterial blood samples were taken before and after an injection of sodium cyanide (8.4 mg/kg) and treatment for analyses of blood cyanide, plasma cyanide, thiocyanate and methemoglobin content. Physiologic parameters were monitored in these treated cyanide-poisoned animals. The time course of methemoglobin formation and physiologic parameters were also monitored in animals receiving only 4-DMAP or sodium nitrite. A maximal methemoglobin level was observed at 30 minutes following injection of 4-DMAP, and 60 minutes post injection with sodium nitrite. Volumes of distribution (Vd) of cyanide were calculated from the concentrations of cyanide in blood samples and doses of cyanide injected. Although 4-DMAP forms methemoglobin more rapidly than sodium nitrite, both compounds form methemoglobin quickly enough to provide protection against cyanide poisoning. The protection offered by either compound against the lethal effects of cyanide was potentiated when used in combination with sodium thiosulfate.

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PREFACE

The work reported herein was conducted under U.S. ARMY BIOMEDICAL LABORATORY Protocol PG4-79-1, which was changed to #250-79-001, entitled "A Comparison of 4-dimethylaminophenol (4-DMAP) and Nitrite in the Treatment of Cyanide Poisoning". The data is recorded in CRDEC-CSL notebook # 9918, and in U.S. Army Biomedical Laboratory Notebooks 09-80 and 39-81. The work was initiated in April 1979 and completed in September 1981.

A portion of this work was presented in abstract form (Hawkins, *et al.,* 1981. Federation of American Societies for Experimental Biology).

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INTRODUCTION

Many compounds have been investigated for treatment of cyanide poisoning in animals and man, but opinions vary regarding the antidote of choice among the various treatment compounds, either singly or in combination. A partial listing includes many diverse chemical compounds, such as methylene blue (Hug, 1932; Chen *et al*, 1934), amyl nitrite (Pedigo, 1888; Chen *et al*, 1933; Klimmek *et al*, 1988 a and b; Paulet, 1954), sodium nitrite (Hug and Marenzi, 1933; Chen *et al*, 1934, 1952), cobalt compounds (Evans, 1964; Hillman, 1974; Klimmek *et al*, 1979 a and b), sodium thiosulfate (Chen *et al*, 1934, 1944, 1952), hydroxocobalamin (Mushett *et al*, 1952; Posner, 1976), rhodanese (Clemedson *et al*, 1954, 1955; Frankenberg, 1980; Sorbo, 1951), paraminopropiophenone (Jandorf *et al*, 1946; Rose *et al*, 1947; Bright and Marrs, 1982, 1987), alpha adrenergic blockers (Furukawa *et al*, 1976; Maeda *et al*, 1977), the aminophenolic compounds (Kiese and Weger, 1965; Kiese and Weger, 1969; Christel *et al*, 1977; Marrs *et al*, 1982), a-ketoglutarate (Moore *et al*, 1986), and hydroxylamine (Kruszyna *et al*, 1982; Vick and Froehlich, 1985 and 1988). A complete listing of compounds for prophylactic and antidotal treatment of cyanide poisoning can be found in the *Toxicology of Cyanides*, Ballantyne and Marrs, eds (1987).

A combination of nitrites and sodium thiosulfate has been the major recognized form of therapy for cyanide poisoning in the United States for over half a century. Nitrites convert hemoglobin to methemoglobin, which is thought to compete with cytochrome oxidase for the cyanide ion forming cyanmethemoglobin. Cyanide in the presence of thiosulfate is converted to less toxic thiocyanate in a reaction thought to be catalyzed by the enzyme rhodanese.

Successes were documented with the combined therapy of nitrite plus thiosulfate in cases of human cyanide poisoning (Chen *et al*, 1944, 1952, 1956, and Wolfsie, 1951). However, the value of human therapy is difficult to evaluate since there is the possibility that some of the victims might have survived without treatment (Cope, 1961). Graham *et al*, (1977) concluded from their extensive review of the literature that cyanide poisoning in man has been poorly documented.

The value of nitrites as antidotes for treatment of cyanide poisoning was questioned because of the delayed formation of methemoglobin. Kiese and Weger (1969) reported that the intravenous (iv) administration of the recommended dosage of sodium nitrite (4 mg/kg) in human volunteers produced blood levels of only 6% methemoglobin. Higher dosages were generally not recommended because of detrimental cardiovascular effects.

Studies of the aminophenols were initiated to find a compound which produced methemoglobin more rapidly than sodium nitrite without the latter's undesirable side effects. One compound that received considerable attention was 4-dimethylaminophenol (4-DMAP). According to Lorcher and Weger (1971), a blood level of 30-40% methemoglobin was required to successfully treat cyanide poisoning. The compound, 4-DMAP, protected dogs against the lethal effects of 3 x LD50 potassium cyanide. The compound had no effect on blood pressure like that observed following administration of the nitrites.

At the time this study was initiated, there was concern about the adequacy of treatment following exposure of troops to cyanide during chemical warfare. Inhalation of amyl nitrite and intravenous administration of sodium nitrite and sodium thiosulfate were considered impractical for treatment of large numbers of exposed victims under field conditions. Since the administration of 4-DMAP intravenously, and especially by intramuscular injection, produced methemoglobin quite rapidly in the dog, further study of this compound was warranted. Therefore, experiments were undertaken to compare blood concentrations of methemoglobin following injection of iv sodium nitrite and im 4-DMAP in a non-human primate. The study was also to compare the two compounds in limiting mortality, with and without sodium thiosulfate, in animals receiving lethal amounts of sodium cyanide. The volume of distribution (Vd) of cyanide was also calculated.

METHODS

Animals. The animals used in this study were male cynomolgus monkeys (Macaca fascicularis) weighing from 2.9 to 6.2 kg. The animals were housed in individual cages in a monkey colony room and were fed Ralston Purina high protein monkey chow, apples, and allowed water ad libitum. The room was maintained at 20-22° C, relative humidity of 50 $\pm$ 4.10%, on a 12-hr light/dark cycle with no twilight. The monkeys were fasted overnight before an experiment.

Surgery. The animals were anesthetized with an iv injection of pentobarbital sodium (30 mg/kg). Additional injections were administered as required. The animals were intubated with a Swinnex #13 endotracheal tube with an inflatable cuff. They were then placed on a temperature controlled surgical table and maintained in a supine position with loosely restrained extremities.

The femoral artery and vein were isolated utilizing aseptic techniques. Sterile polyethylene catheters (P.E. #160) were inserted in the femoral artery for collection of samples of arterial blood and recording of blood pressure. The femoral vein was cannulated with P.E. #190 for all injections. All glassware, surgical instruments and draping sheets were sterile. Sterile surgical techniques were used throughout the experiments.

Cardiopulmonary measurements. Blood pressure, heart and respiratory rates were monitored continuously and recorded on a Narco 6-B Physiograph. Air flow was monitored by attaching a Fleisch pneumotachograph to the endotracheal tube, while respiratory movements were recorded from a Narco impedance pneumograph. Electrocardiograms were recorded on a Hewlett Packard 1515 B cardiograph with adhesive discs and leads from the four limbs and chest. A thermistor probe was used to record rectal temperature on a Yellow Springs Instrument, Model 42SC. Solutions of sodium cyanide, sodium nitrite, 4-DMAP and sodium thiosulfate were freshly prepared each day, and were sterilized by passage through millipore filters before injection. To reduce the possibility of clots in blood samples, each animal received Lyphomed (heparin, 750 mg/kg, iv). A volume of heparinized saline (3 ml 1:1000 heparin/100 ml saline), equal to the volume of withdrawn blood, was injected at each sampling time. Blood samples were drawn prior (-10 and -5 minutes) to the time of injection of sodium cyanide (zero time), subsequently at 5, 10, 15, 30, 45 and 60 minutes and at hourly intervals for 2-4 hours. All samples were analyzed immediately.

The blood samples were analyzed at 37° C for blood gases and pH on Instrumentation Laboratory 513 or 813 blood gas analyzers. The samples were also analyzed for hemoglobin, microhematocrit, blood and plasma cyanide, thiocyanate and methemoglobin. Methemoglobin and total hemoglobin were assayed by the method of Groff *et al.* (1974). Blood and plasma cyanide were measured by the automated method of Groff *et al.* (1985). The measurement of blood cyanide includes the cyanide located primarily within the erythrocytes in the form of a cyanmethemoglobin complex along with cyanide in unbound form in plasma. Thiocyanate was assayed by the method of Butts *et al.* (1974).

Experimental Procedures. The study consisted of three phases: 1) a comparison of time-duration curves of methemoglobin by 4-DMAP or sodium nitrite, 2) determination of the LD50 dosage of sodium cyanide and 3) treatment of four groups of cyanide-poisoned animals with sodium nitrite or 4-DMAP, and with sodium nitrite or 4-DMAP in combination with sodium thiosulfate. The groups of animals and test dosages used are summarized in Table 1.

Table 1. Experimental Groups

		Treatment			
Group	Number of Animals	NaCN (a) mg/kg	4-DMAP (b) mg/kg	$NaNO_2$ (c) mg/kg	$Na_2S_2O_3$ (d) mg/kg
1	5	---	5	---	---
2	5	---	---	20	---
3	6	8.4	---	20	---
4	6	8.4	5	---	---
5	6	8.4	---	20	179
6	6	8.4	5	---	179
7	23	1.89-3.78	---	---	---

a) Bolus of sodium cyanide iv.
b) 4-DMAP im injection in triceps.
c) Two minute iv infusion of sodium nitrite.
d) Five minute iv infusion of sodium thiosulfate.

Antidotal treatment with either 4-DMAP (Farbwerke Hoechst, Frankfort a. M.) or sodium nitrite (Chemical Manufacturing Division, Fairlawn, N. J.) was begun one minute after the iv injection of a lethal dose of sodium cyanide (Mallinckrodt, Inc., Paris, Kentucky) (8.4 mg/kg in 2.0 ml 0.9% saline). Sodium nitrite (20 mg/kg in 2 ml distilled water, iv) was infused over a two-minute period, while 4-DMAP (5 mg/kg in 1.0 ml of ascorbic acid and bicarbonate solution) was injected as a bolus into the triceps. The 4-DMAP was dissolved in 1.0 ml of stock solution (10 ml containing 0.005 g ascorbic acid (Hartman Leddon Co., Philadelphia, Pa.) and 0.02 g sodium hydrogen carbonate (Allied

Chemical, Industrial Chemicals Division, Morristown, N. J)). Some of the animals receiving either sodium nitrite or 4-DMAP were also infused iv with sodium thiosulfate (Mallinckrodt, Inc. Paris, Kentucky) (178.6 mg/kg in 5.0 ml distilled water) over a five minute period, one minute after drawing the one hour blood sample. None of the cyanide poisoned animals received artificial ventilation or mechanical stimuli to initiate breathing.

At the end of an experiment the catheters were removed from surviving animals, and the femoral artery and vein were ligated above the level of catheter insertion. The incision was sprayed with Topazone, sutured and sprayed again. The animals were returned to their cages and survival was recorded at 24 hours post cyanide. Non-survivors were necropsied.

Statistical Approach. The up-down method of Dixon and Mood described in Finney (1971) was used in determining the lethal dose of cyanide in monkeys. The results of this approach were analyzed by the method of Litchfield and Wilcoxon (1949).

A two-way analysis of variance was performed on the baseline values for the physiological and biochemical parameters to determine if control values at -10 and -5 minutes were the same and if the baselines were the same for all groups. If an overall time effect was significant, then a Dunnett's test was used to compare each post injection level against baseline.

Treatment groups were compared with a two way (time and group) analysis of variance on all parameters. Changes from baseline were used for all parameters except cyanide related ones, since there would be no cyanide concentration at baseline. Baseline cyanide could not be measured since the analyses were performed at a lower sensitivity range to accommodate the expected high blood cyanide values after cyanide administration. If a significant interaction (time by group) was observed, groups were compared at each time point using a Newman-Keuls multiple comparison test (Winer, 1962).

Statistical significance was declared at the $p<0.05$ level.

RESULTS

Formation of Methemoglobin by Sodium Nitrite or 4-DMAP in Animals. Figure 1 illustrates the time duration curves of concentrations of methemoglobin following the injection of im 4-DMAP and iv sodium nitrite in the anesthetized monkey. Maximal production of methemoglobin was attained at 30 minutes and at 60 minutes with 4-DMAP and sodium nitrite, respectively. A comparison of formation of methemoglobin shows the more rapid onset to a maximal concentration by 4-DMAP. The half time to peak for 4-DMAP was 3 minutes and 10 minutes for sodium nitrite, even though the latter compound was given intravenously. The decay phases suggest that the reduction of methemoglobin to hemoglobin during the 2- to 4-hour period was similar for the two compounds.

Lethality of Sodium Cyanide. The intravenous LD50 of sodium cyanide determined in 23 anesthetized monkeys was 2.8 (95% confidence limits of 2.3-3.4) mg/kg (Fig. 2). The mean blood cyanide concentrations following injection of the several dosages of sodium cyanide in these animals are shown in a semilogarithmic plot versus time in Figure 3. Curves with biphasic disappearance patterns were observed; an initial rapid rate of elimination was followed by much slower rates of elimination. Table 2 shows the volume of distribution of cyanide (Vd in l/kg) calculated from the amount of cyanide (mg) injected / cyanide concentration (mg/l at t=0) / body weight (kg). The volume of distribution calculated from the highest blood value (at one minute in Figure 3) was found to be approximately 0.20 l/kg (Vd_1). A Vd_2 of cyanide (0.264 l/kg) was calculated from the intercept using points during the 3 to 15 minute period. The Vd_3 in poisoned animals after 4-DMAP/nitrite was also calculated using the maximum concentration (between 0.5 and 1.0 hr.) of cyanide measured in the treated animal. The Vd_3 values were much lower than Vd_1 and Vd_2.

Table 2. The Apparent Volume of Distribution (Vd) of Cyanide

A.	NaCN mg/kg	# of Animals	Vd1 L/kg	Vd2 L/kg
Cyanide	1.89	1	0.190	0.250
	2.38	4	0.189 ± 0.011	0.276 ± 0.078
	2.67	6	0.200 ± 0.030	0.254 ± 0.059
	3.00	3	0.227 ± 0.012	0.319 ± 0.046
	3.37	4	0.211 ± 0.014	0.265 ± 0.042
B.	**NaCN mg/kg**	**# of Animals**	**Vd3 L/kg**	
Cyanide + Sodium Nitrite	8.4	10	0.057 ± 0.005	
Cyanide + 4-DMAP	8.4	10	0.052 ± 0.004	

Calculated from data (A) obtained in monkeys receiving cyanide only (Figure 3), and from data (B) in monkeys receiving both cyanide and treatment (Figures 10, 11, 12 and 13).
Vd1 Calculation based on the highest blood concentration of cyanide at 1 minute;
Vd2 Calculation based on intercept obtained by regression analysis of values during the 3 to 15 minute interval; and Vd3 Calculation based on the highest blood concentration between 30-60 minutes post-injection of cyanide.
Mean baseline values ± S.D.

Physiological Observations. The mean physiological responses of circulatory and respiratory parameters in two groups of animals receiving either sodium nitrite (Figures 4) or 4-DMAP (Figure 5) and in four cyanide poisoned, treated groups are shown in

Figures 6-9. The initial values are plotted beginning at 3 minutes in Figures 4-5 and at 5 minutes in Figures 6-9. With the exception of pH, the mean values are expressed as % change from control values which were set at a baseline of 100%. In all figures n in parentheses = number of animals alive during the time period when blood samples were drawn. Error bars have been eliminated to show the pattern of responses in Figures 4-9.

Animals Injected with Sodium Nitrite or 4-DMAP. A comparison of results in Figures 4 and 5 of animals treated with sodium nitrite or 4-DMAP alone shows that the most striking physiologic change occurred in blood pressure. Blood pressure dropped precipitously in the nitrite group to a significantly lower level, which was maintained without change for two hours, while only a minor fluctuation occurred in the 4-DMAP group. Animals in both groups showed a transient peaked increase in pO_2 within minutes after injection of each antidote. pO_2 was significantly different from the baseline values at all time points (except 10 min) to the 60th minute in the 4-DMAP group; pO_2 in the nitrite group differed from baseline at 3 minutes and at 30 minutes through the two-hour point. In the 4-DMAP treated group, pCO_2 was less than baseline at the 180- minute point, while in the nitrite group, pCO_2 was less than baseline at the 10, 15, 60, 120 and 180 minute points. Heart rate was significantly higher than baseline values at all time periods in the nitrite animals, and differed from baseline values at 30, 60, 120 and 180 minutes in the 4-DMAP animals. The respiratory rate in the nitrite group differed from baseline beginning with the hourly measurements, while the respiratory rate of the 4-DMAP group was not significantly different from baseline. There were no significant acid base or ventilatory changes in animals receiving either 4-DMAP or sodium nitrite only (See Appendixes A and B and Figures 6 and 7).

Cyanide-poisoned Animals Treated with Either Sodium Nitrite or 4-DMAP, with and without Sodium Thiosulfate. Blood gas and pH data are shown in Appendixes C-F, and the cardiorespiratory data beginning at 5 minutes post injection are shown in Figures 6-9. Immediately after cyanide all animals responded with a period of hyperventilation (not shown), approximately 30 to 40 seconds in duration, followed by a prolonged period of apnea 1 to 3 or more minutes in duration interrupted by occasional gasps. Following apnea, spontaneous breathing in the treated animals resumed with a gradual increase in rate. During the period of hyperventilation a transient increase in blood pressure was also observed followed immediately by bradycardia and hypotension. The electrocardiogram showed cardiac irregularities which included arrhythmias and complex wave changes.

The mean baseline values for these four poisoned groups (Figures 6, 7, 8, and 9) of animals treated with antidotes were not different from the baseline values in the two groups (Figures 4 and 5) receiving nitrite or 4-DMAP. The odor of cyanide was detectable in the exhaled air of the animals immediately following intravenous injection. Pupillary dilation was a common event. No convulsions were noted in these monkeys anesthetized with sodium pentobarbital. No urination or defecation was observed following the administration of cyanide.

The mean physiological responses in each of the four poisoned groups receiving antidote showed similar patterns in response to cyanide. The transient pO_2 peaks, observed in animals which received either nitrite or 4-DMAP alone, were also observed in the poisoned treated animals. Differences in arterial pO_2 occurred between groups only within the time periods of 5 to 30 minutes. Few significant differences in pCO_2 between treated poisoned groups were observed. Each of the four groups showed an onset of hyperventilation which persists and which was compensated by systemic acidosis. Few significant differences were noted in pH between groups. Although initially highly variable from group to group, blood pressure remained below baseline values. The slowed heart rate observed shortly after injection of cyanide returned to the baseline rate in about 15 minutes. The heart rate of the four groups were approximately equal to each other at times 15, 30, 45, 60, 120 and 180 minutes. Breathing rates showed no group differences, but higher rates were noted with time.

Blood Concentrations of Blood and Plasma Cyanide, Methemoglobin and Thiocyanate in Poisoned, Treated Animals. The mean arterial blood concentrations of blood and plasma cyanide, methemoglobin and thiocyanate in poisoned animals treated with each of the four regimens are shown in Figures 10, 11, 12 and 13. The concentrations of methemoglobin and thiocyanate in baseline blood samples from 24 animals were 25.6 SD $\pm$ 6.0 and 18.9 SD $\pm$ 5.6 umol/l, respectively.

The results shown in Figures 10 and 11 indicate that plateaus of mean blood cyanide levels were reached between 30 and 60 minutes post injection of cyanide after which concentrations decreased slowly. In all groups, mean blood thiocyanate concentrations increased steadily, in linear fashion, throughout the first hour post cyanide. Shortly after treatment of the poisoned animals with 4-DMAP, a single maximal mean methemoglobin concentration was observed, whereas a biphasic curve was obtained after administration of nitrite. In the nitrite groups there was an early smaller peak followed by a decline with subsequent return to a higher more sustained methemoglobin level. The mean maximal methemoglobin concentrations in the poisoned, treated animals never exceeded 300 umol/l (15% of total hemoglobin) during the first hour post injection. At the five minute point approximately 30-50 umol/l of cyanide were measured in the plasma. The concentration of plasma cyanide decreased slowly with time. Comparison of the Vd in Table 2 showed that the smallest Vd ($Vd_3 \approx 0.055$ l/kg) was observed in cyanide-poisoned animals after treatment with sodium nitrite or 4-DMAP.

Figures 12 and 13 show the biochemical responses to treatment of poisoned animals with sodium nitrite or 4-DMAP, plus delayed treatment at one hour with sodium thiosulfate. It should be noted that the response patterns are parallel in all four treatment groups during the first sixty minutes post cyanide. However, the influence of sodium thiosulfate on arterial blood concentrations of blood and plasma cyanide, methemoglobin and thiocyanate can be seen by comparison of Figures 12 and 13 with Figures 10 and 11 (treatment of cyanide poisoning with either nitrite or 4-DMAP alone).

The marked downward shift in the slope of the blood cyanide curves upon administration of thiosulfate clearly demonstrated accelerated loss of cyanide from blood (presumably from cyanmethemoglobin). The rate of removal of cyanide from plasma also increased at the same time. The t1/2 (in hours) for blood cyanide in the treated groups was as follows: nitrite (9.1), nitrite + thiosulfate (0.91), 4-DMAP (3.93), and 4-DMAP + thiosulfate (0.73). Simultaneously, there was an abrupt increase in the concentration of thiocyanate which increased steadily throughout the experimental time frame. The increasing concentration of thiocyanate was accompanied by a gradually increasing level of methemoglobin.

The comparative protective effects of the four treatment regimens against cyanide poisoning and survival times are shown in Table 3. S indicates that the animal survived for twenty-four hours. Four animals in group 3 (nitrite treated) and three animals in group 4 (4-DMAP treated) died within 24 hours post cyanide. All animals survived when treated with nitrite and sodium thiosulfate (group 5). Five of six animals survived after treatment with 4-DMAP plus sodium thiosulfate (group 6). The plasma cyanide level at five minutes post cyanide exceeded 200 umol/l in the non-survivor and may account for the early death of the animal in this group. It must also be noted that the animals treated with 4-DMAP survived for at least one hour post cyanide, which should provide sufficient time for delayed treatment with sodium thiosulfate. We are unable to account for the differing survival rates in the two groups receiving nitrite since all animals in the nitrite-thiosulfate group survived at least one hour, whereas in the nitrite treated poisoned group, three deaths occurred within the first hour post cyanide.

Table 3. Survival Times in Cyanide Poisoned and Treated Animals

Animal	Group 3 $NaNO_2$	Group 4 DMAP	Group 5 $NaNO_2 + Na_2S_2O_3$	Group 6 DMAP + $Na_2S_2O_3$
1	S	S	S	S
2	10 min	< 24 hours	S	S
3	S	150 min	S	S
4	32 min	S	S	S
5	54 min	S	S	S
6	150 min	119 min	S	7 min

S = survivor

Necropsies. There were no obvious findings that would differentiate animals dying as a result of cyanide poisoning from animals dying from other causes.

DISCUSSION

The present study compared the formation and duration of methemoglobin following injection of 4-DMAP or sodium nitrite alone in the cynomolgus monkey. The slow transformation of hemoglobin to methemoglobin by nitrite was consistent with the findings in dogs reported by Kiese and Weger (1969). A maximal concentration of 824 umol/l methemoglobin was produced by 5 mg/kg 4-DMAP, but only 632 umol/l methemoglobin was attained with 20 mg/kg sodium nitrite. These concentrations correspond to methemoglobin levels of approximately 43% and 38% of total hemoglobin for 4-DMAP and sodium nitrite, respectivley. Kiese and Weger, 1969, reported that 20 mg/kg sodium nitrite was required to also produce 40% methemoglobin in the dog. Chen and Rose, 1952, recommended 300 mg as the dosage of sodium nitrite for cyanide poisoning in the adult human. This is approximately 4 mg/kg for a man weighing 75 kilograms. In human volunteers, this dosage was reported to produce 6.0% methemoglobin which was accompanied by a significant lowering of blood pressure and often orthostatic collapse. Accordingly, administration of larger amounts of nitrite are not recommended for treatment of cyanide poisoning in man.

The physiologic effects of these two methemoglobin inducers are described in the non-poisoned monkey. The methemoglobin former, 4-DMAP, had little effect on blood pressure, breathing and heart rate in the monkey. In contrast, sodium nitrite lowered blood pressure for long periods of time. Both compounds produced a transient pO_2 peak which appears to be the result of released oxygen from erythrocytes during the conversion of hemoglobin to methemoglobin (Klimmek *et al*, 1979a). The pO_2 then fell below baseline values and proceeded to a minimum level which approximated the time of maximal methemoglobin formation. The pO_2 peaks observed in animals with methemoglobinemias produced by 4-DMAP or sodium nitrite were also observed in cyanide-poisoned animals following treatment.

The distribution of cyanide following intravenous administration is rapid due to its great diffusion capability. In the monkey receiving only sodium cyanide, the highest blood cyanide concentration was attained at one minute post injection with a subsequent rapid decrease during the next 15 minutes (Figure 3). Blood cyanide then continued to decrease more slowly in linear fashion up to 120 minutes. *In vitro* studies have shown that the addition of cyanide to blood results in rapid distribution and accumulation of cyanide within red blood cells (McMillan *et al*, 1982). Other studies have shown that following addition of cyanide to blood, 80-90% of recovered cyanide was located in the erythrocytes (Vesey and Wilson, 1978). If the results from *in vitro* studies apply equally to cyanide injections in an animal, distribution of cyanide into the erythrocytes should be accompanied by simultaneous movement of cyanide from the circulation to body tissues and fluid compartments. Cyanide also rapidly enters the various excretion pathways. For example, the odor of cyanide was briefly detectable in exhaled air of the monkey immediately following administration of cyanide. Less than 1% of injected cyanide was lost via the lungs in cyanide-poisoned dogs pretreated and infused with sodium thiosulfate (Sylvester *et al*, 1983).

However, in the cyanide poisoned monkey treated with nitrite or 4-DMAP, a maximal concentration of cyanide in blood was not reached for 30-60 minutes after injection of cyanide. Note that a peak blood cyanide concentration was probably attained immediately after injection, but was not detected due to delayed sampling for 5 minutes. Following the injection of cyanide and the antidote, the blood cyanide level was increasing as shown in Figures 10-13. During this interval of approximately 5-15 or more minutes methemoglobin was being formed, simultaneously there was an ongoing return of cyanide from tissue cells and fluid compartments into blood thereby minimizing the effects in tissues sensitive to cyanide. The low concentration of cyanide in plasma as early as 5 minutes in Figures 10-13 also emphasizes the effectiveness of methemoglobin in complexing unbound cyanide.

Gregersen *et al.*, 1959, reported a blood volume of 55 ml/kg for the rhesus monkey. An assumption was made that the blood volume for the cynomolgus monkey was equal to that in the rhesus monkey. The amount of injected cyanide was divided by the blood volume of 55.0 ml/kg to obtain the cyanide concentration. There was good agreement between maximal concentration of cyanide measured in blood at 30-60 minutes and the predicted cyanide concentration (Table 4). The recovery of cyanide in blood (Table 4) was found to be approximately 10% higher in the 4-DMAP treated animals compared with animals receiving sodium nitrite. These data provide support that with formation of cyanmethemoglobin, blood becomes a circulating "depot" of cyanide as cyanide proceeds through the various excretory pathways.

The Vd_2 (0.264 SD $\pm$ 0.042 l/kg) of cyanide in monkeys (Table 2) was greater than that (0.209 l/kg) obtained in beagle bitches by Bright and Marrs (1988). A comparison of the plots of blood cyanide concentrations indicates that the initial elimination rate in the monkey was much faster than in the dog. In the monkey the slower phase of elimination began at approximately 15 minutes compared to 75 to 80 minutes in the beagles. The value for Vd_1 may represent incomplete distribution of cyanide at one minute post injection.

Table 4. Comparison of Measured and Calculated Concentration of Cyanide in Blood

# of Animals	Treatment Group	Measured Mean uM/l	Calculated Mean uM/l	% Recovery Mean
4	3	2920	3136	93.1
6	5	3060	3132	97.7
5	4	3295	3133	105.2
5	6	3396	3135	108.3

Calculated concentration based on an iv injection of 8.4 mg/kg sodium cyanide and an assumed blood volume (see text).

The Vd of cyanide was calculated for all animals after receiving treatment with sodium nitrite or 4-DMAP, with and without sodium thiosulfate. The maximum blood cyanide values from 20 monkeys were used for this calculation. A Vd_3 of 0.055 SD $\pm$ 0.005 l/kg (55.0 ml/kg) was obtained; this is equivalent to the blood volume which indicates that all of the injected cyanide was located in the blood. By comparison the Vd in the untreated poisoned animal (Vd_2) was approximately 0.264 l/kg. It must be noted that animals in this group were injected with lower dosages of cyanide which ranged from 1.89 - 3.78 mg/kg. In the presence of a methemoglobinemia, the volume of distribution of cyanide (0.055 l/kg) in the poisoned animal was reduced due to redistribution and confinement of cyanide within the circulating blood volume. The volume of distribution is probably even smaller than the latter value in these animals since cyanide is restricted to or contained only within the erythrocyte volume.

Thus, in poisoned animals treated only with methemoglobin inducers, blood cyanide was lowered gradually during conversion to thiocyanate. A linear increase in thiocyanate formation was observed in animals treated with either 4-DMAP or sodium nitrite. A gradual reduction in plasma cyanide was also observed with time.

However, in monkeys treated with sodium thiosulfate in the presence of a methemoglobinemia, the source of additional sulfur altered the blood picture. One effect of sodium thiosulfate was to accelerate the rate of thiocyanate formation (Figures 12 and 13). The downward slopes of blood and plasma cyanide were also changed indicating accelerated loss of cyanide from blood and plasma, respectively. There was a faster fall of blood cyanide in animals treated with methemoglobin generators and thiosulfate, compared with those which received only sodium nitrite or 4-DMAP. Finally, increasing levels of methemoglobin were observed; presumably a result of the loss of cyanide from cyanmethemoglobin with time.

Part of the protection afforded by the nitrites has been ascribed to some mechanism other than that of methemoglobin formation (Way *et al.*, 1984, Vick and Froehlich, 1985, Baskin *et al.*, 1986). It was suggested that the vasogenic effects of the nitrites may be involved. Only a slight decrease in blood pressure was observed in the animals receiving 4-DMAP alone. However, a comparison of blood pressure between animals (non-poisoned *vs* poisoned) receiving 4-DMAP suggested that cyanide might be contributing to a lowered, but not significantly different, blood pressure. The drop in blood pressure caused by sodium nitrite doesn't seem to be important in enhancing survival after exposure to cyanide. In fact the potentiation of survival with sodium thiosulfate seems to be related to the prevention of a large decrease in blood pressure in Figures 8 and 9. It still appears that the primary mechanism common to sodium nitrite or 4-DMAP during the early stages of methemoglobin formation was the complexing of cyanide by methemoglobin. A similar conclusion was reached by Klimmek and Krettek, 1988a, who used amyl nitrite in dogs poisoned with potassium cyanide and were likewise unable to find another antidotal mechanism other than formation of methemoglobin.

As stated earlier, the formation of thiocyanate is believed to be the result of a combination of cyanide and sulfur in the presence of an enzyme, presumably rhodanese (EC 2.8.1.1., thiosulfate cyanide sulfurtransferase) (Sorbo, 1951). Because of its great rhodanese content and activity, the liver has usually been designated as the primary site for conversion of cyanide to thiocyanate. Since the studies of DeDuve *et al.*, 1955 and Schubert and Brill, 1968 showed that rhodanese is intimately associated with the mitochondria of liver cells, the reaction between cyanide and sulfur/thiosulfate appears to be restricted within mitochondria (cytochrome oxidase) during the formation of thiocyanate. Sulfur/thiosulfate must therefore penetrate the cell membrane of blood capillaries, of liver cells as well as the double layered membrane surrounding mitochondria. This reaction has not been satisfactorily explained due to the limited penetration of sodium thiosulfate through cell membranes (Himwich and Saunders, 1948). Even the primary role of the liver in cyanide metabolism has been questioned since cyanide metabolism was affected little by removal of a major portion of the liver or following damage to liver cells (Rutkowski *et al*, 1986).

Involvement of the liver in cyanide metabolism may also be questioned from the experiments of Sylvester *et al*, 1983. In cyanide poisoned animals pretreated with, and infused with thiosulfate, most of the conversion of cyanide to thiocyanate was reported to occur quite rapidly. The protective effect of thiosulfate took place within the circulatory system, either in blood or tissue areas in close proximity to blood. The authors suggested that detoxification of cyanide in blood implies cyanide metabolism by sulfur transferases other than rhodanese.

Apparently the metabolism of cyanide to thiocyanate is not restricted to the mitochondria located in the liver (or kidney). Although their roles are not well understood, a number of enzymes (sulfur transferases) located in erythrocytes have been implicated in cyanide metabolism (Westley, 1981). However, little or no rhodanese was present in blood according to Himwich and Saunders, 1948. Several *in vitro* studies suggest involvement of blood in cyanide metabolism (Coltori and Giusti, 1955; Gee *et al*, 1987; McMillan and Svoboda, 1982; and unpublished observations in our laboratory). Recently the optimum conditions for measuring rhodanese activity in human erythrocytes were established (Vazquez *et al*, 1987). However, the concept of cyanide metabolism within erythrocytes is not in accord with the work of Piantadosi and Sylvia (1984). Their experiments in rats, in which blood was replaced with fluorocarbons, showed that cyanide metabolism was still ongoing in bloodless animals (in the absence of erythrocytes and serum proteins).

The lethal effects of cyanide (2.5 mg/kg) in anesthetized dogs were successfully prevented by treatment with only methemoglobin formers, hydroxylamine or 4-DMAP (Vick and Froehlich, 1988). Apparently, at this dosage, the dog does not require an exogenous source of sulfur as sodium thiosulfate for treatment. However, the synergistic effect of a combination of sodium thiosulfate with a methemoglobinemia by either 4-DMAP or sodium nitrite was required to insure survival of monkeys injected with larger lethal amounts of cyanide (8.4 mg/kg). A species difference may exist. The dog has been reported to require days to excrete cyanide in the form of thiocyanate (Mukerji and Smith, 1943), which may be related to the low rhodanese content in dogs. The rhodanese content in tissues of the dog were reported to be much lower than those in the rhesus monkey (Himwich and Saunders, 1948).

In summary, these studies show these methemoglobin formers to be equally effective in treating cyanide poisoning in monkeys, especially in combination with sodium thiosulfate. Although there was no decrease in blood pressure following injection of 4-DMAP alone, these studies reveal many other similarities, in physiologic as well as biochemical responses, between sodium nitrite and 4-DMAP when used to treat cyanide poisoned animals.

Figure 1.

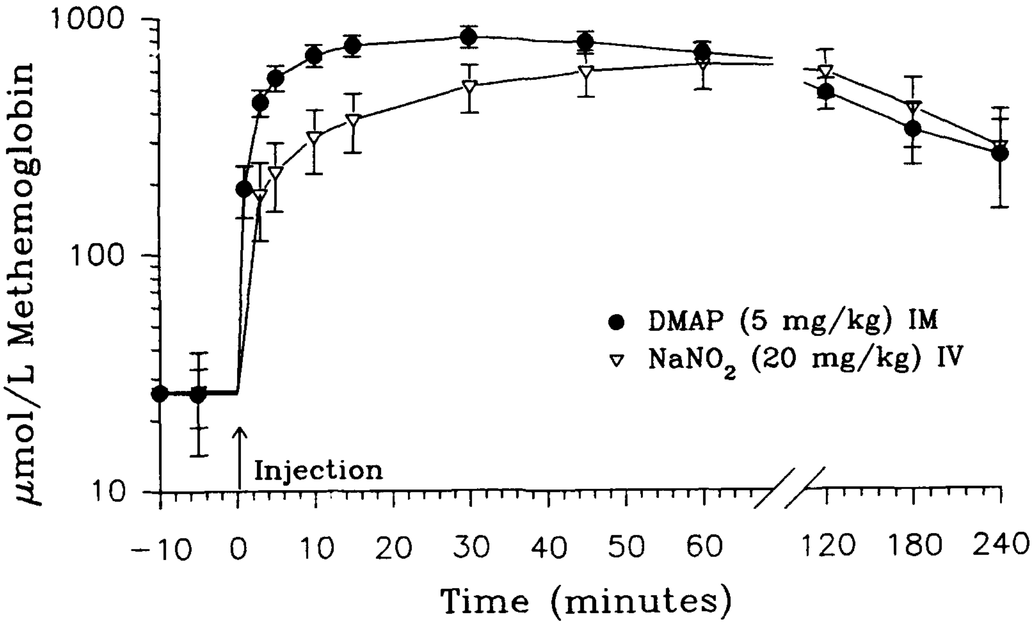


Figure 2.

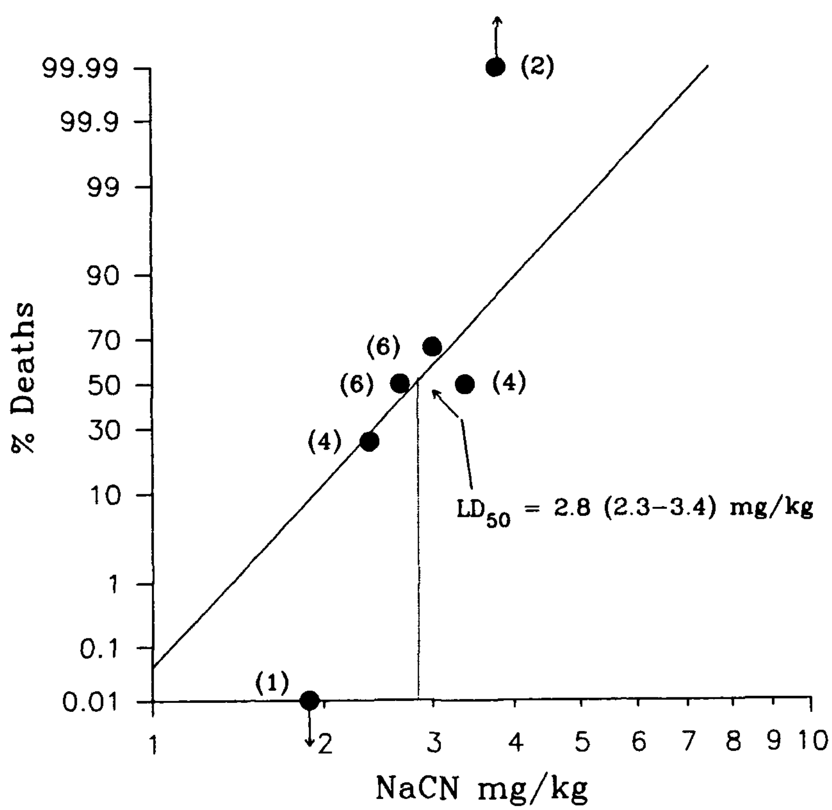
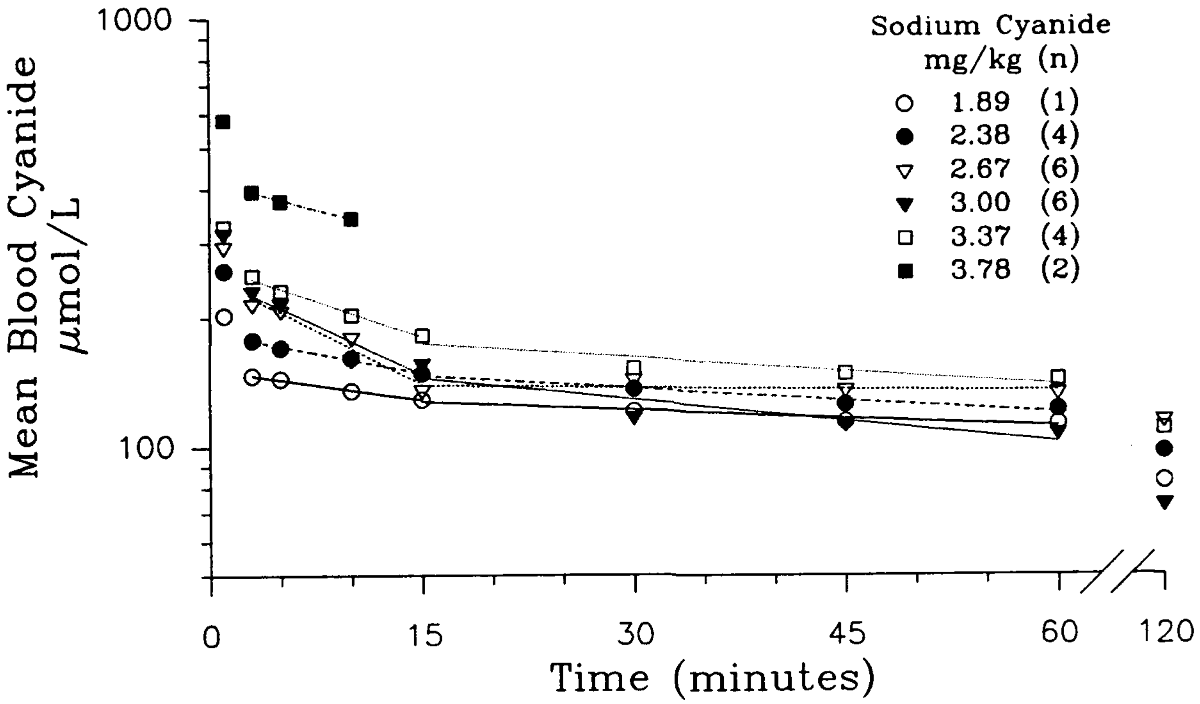


Figure 3.



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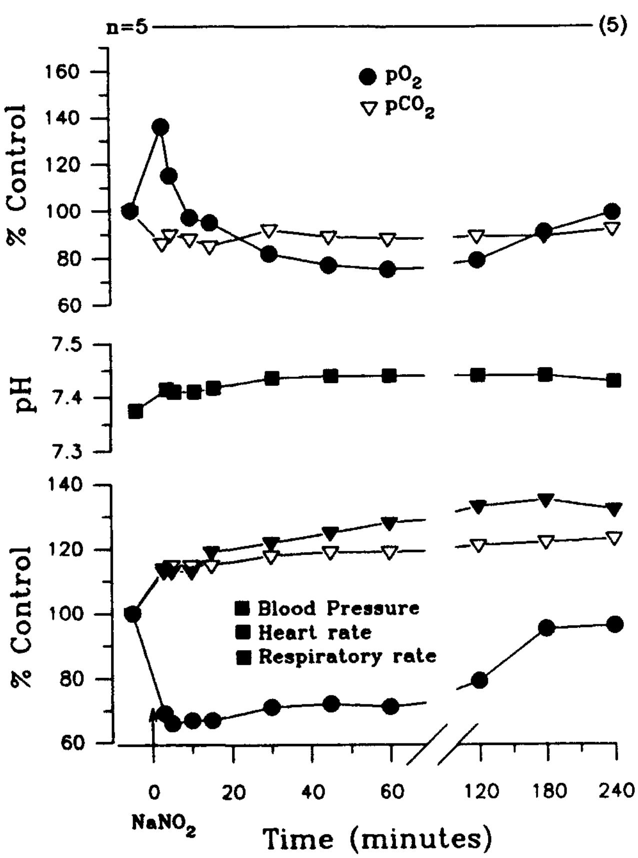

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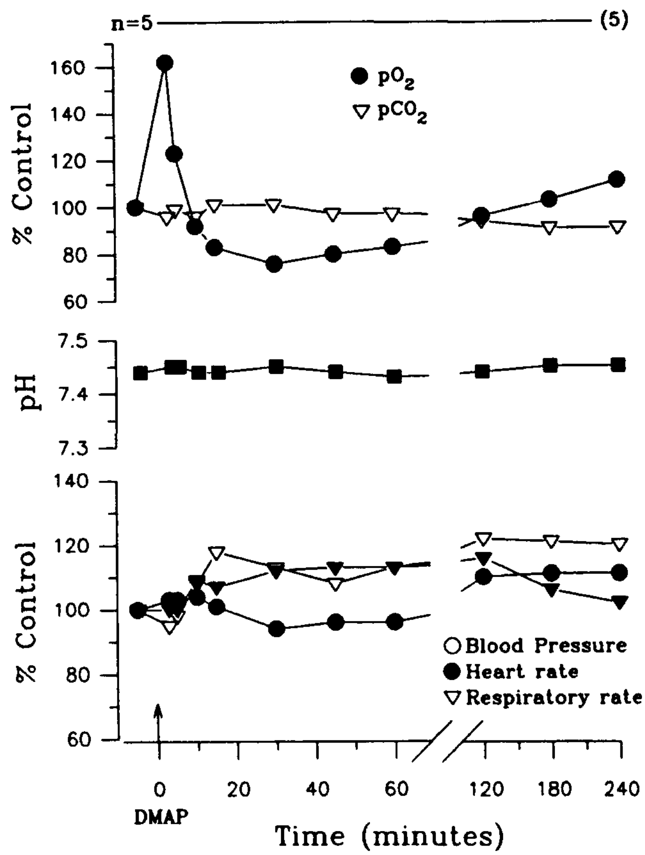

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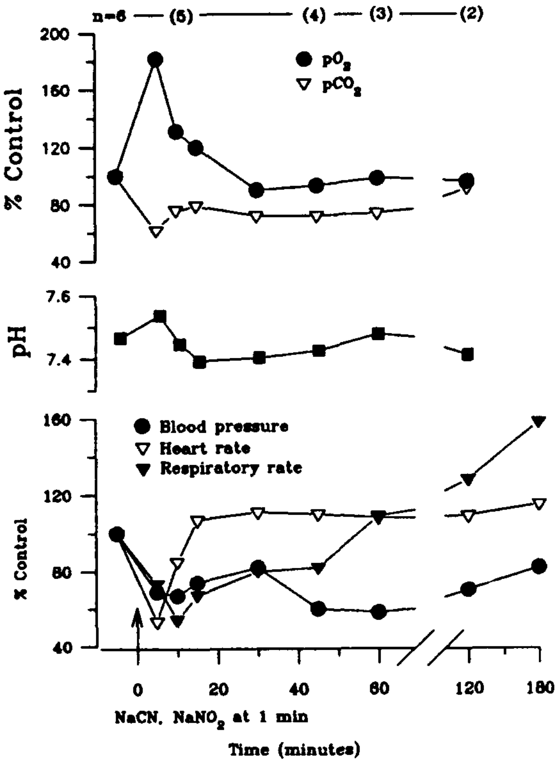

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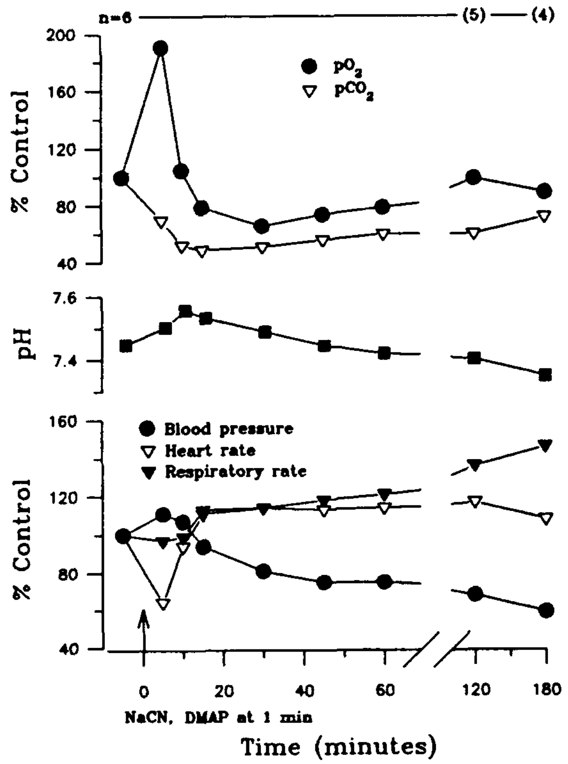


Figure 8.

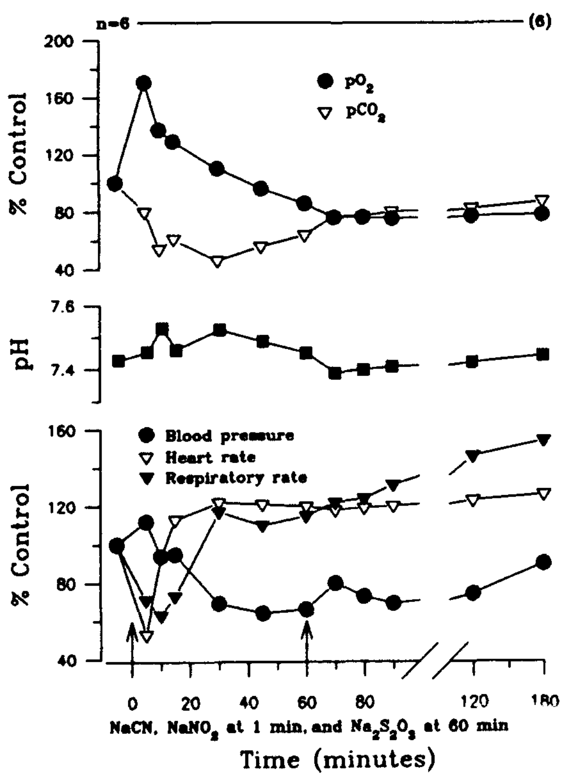
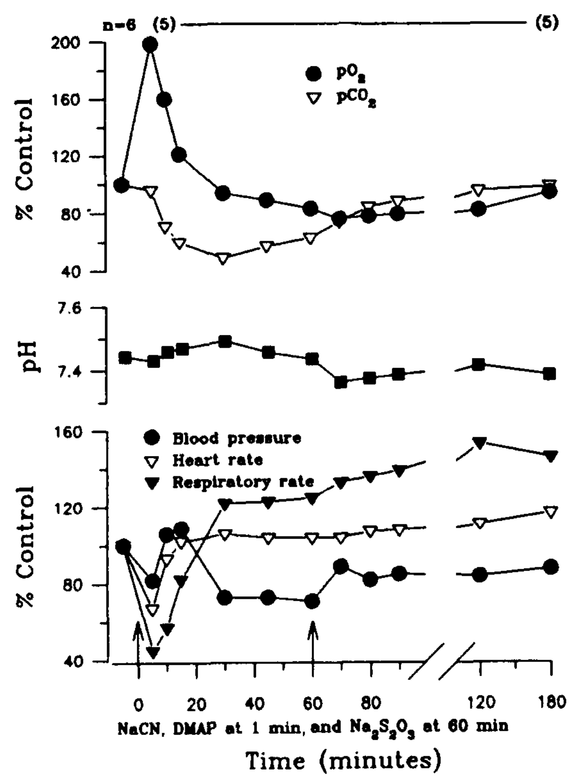


Figure 9.



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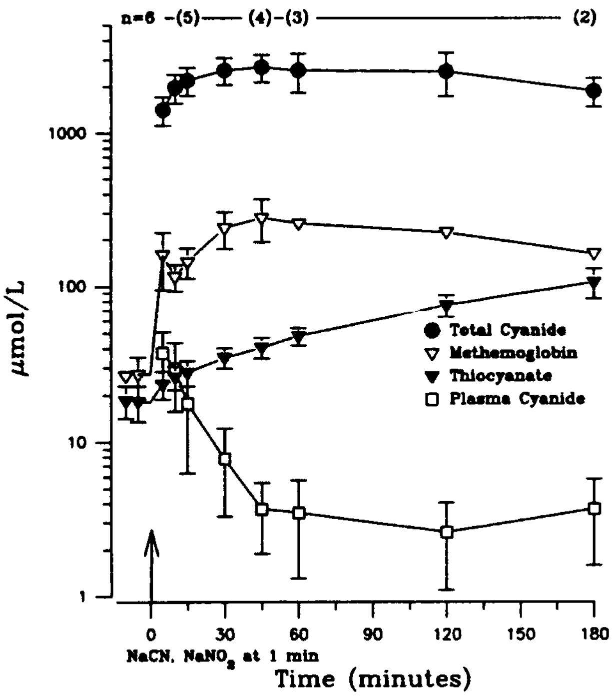


Figure 11.

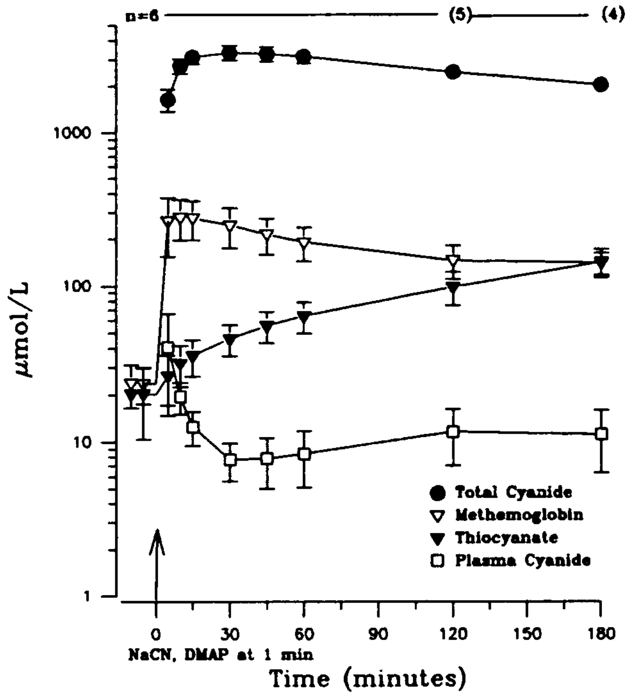


Figure 12.

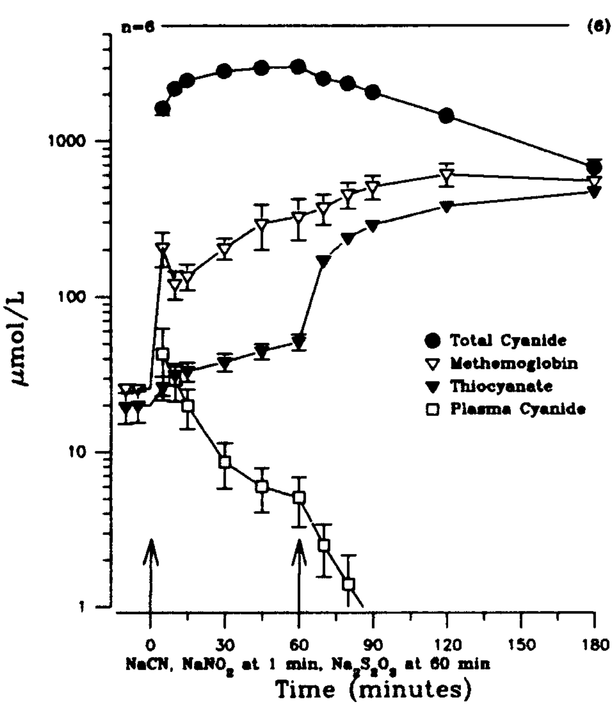
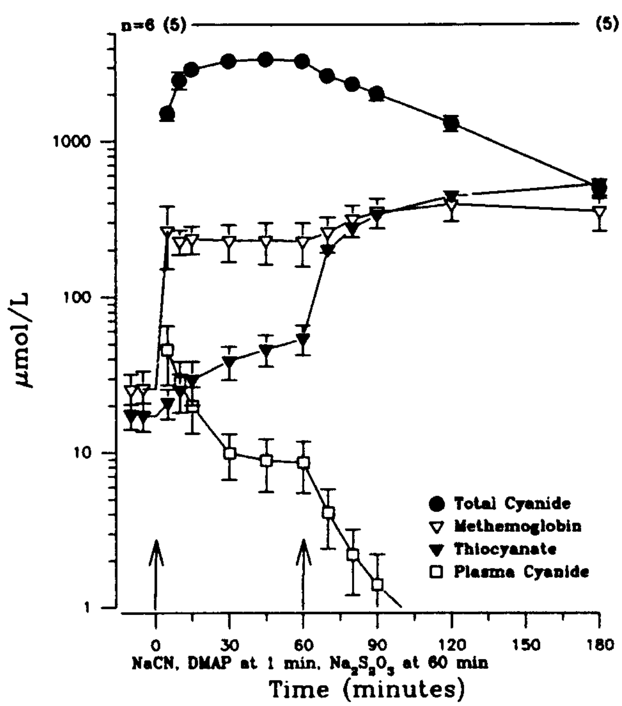


Figure 13.



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APPENDIXES

APPENDIX A. Arterial blood gases and pH in animals receiving 4-DMAP (5 mg/kg im) only. Data are presented as means with ± S.D.

Time in min	pH	pCO_2	pO_2	BE	HCO_3^-	CO_2ct
-10	7.435 ± 0.03	38.7 ± 1.5	92.6 ± 8.6	1.8 ± 2.2	25.4 ± 2.0	26.6 ± 2.1
-5	7.440 ± 0.02	38.4 ± 1.9	93.3 ± 4.6	2.2 ± 2.0	25.7 ± 1.9	27.0 ± 1.9
1	7.440 ± 0.03	36.8 ± 2.8	137.3 ± 19.3	1.4 ± 1.8	24.8 ± 1.8	26.0 ± 1.9
3	7.451 ± 0.03	38.1 ± 1.5	150.6 ± 2.0	2.4 ± 1.8	25.9 ± 1.4	27.1 ± 1.4
5	7.447 ± 0.03	37.0 ± 3.3	114.0 ± 7.1	1.6 ± 1.9	25.0 ± 2.1	26.2 ± 2.2
10	7.442 ± 0.02	38.7 ± 1.3	85.4 ± 8.7	2.3 ± 1.6	25.9 ± 1.4	27.1 ± 1.3
15	7.443 ± 0.02	38.7 ± 2.3	76.7 ± 13.6	2.4 ± 2.1	25.9 ± 2.1	27.2 ± 2.2
30	7.445 ± 0.02	37.4 ± 1.1	70.2 ± 10.1	1.8 ± 1.7	25.1 ± 1.4	26.3 ± 1.4
45	7.437 ± 0.02	37.5 ± 1.5	74.5 ± 10.0	1.2 ± 1.2	24.7 ± 0.8	25.9 ± 0.8
60	7.434 ± 0.1	37.1 ± 1.1	76.7 ± 7.4	0.8 ± 1.6	24.3 ± 1.1	25.4 ± 1.1
120	7.440 ± 0.0	36.3 ± 1.4	89.5 ± 7.0	0.8 ± 1.6	24.0 ± 1.2	25.2 ± 1.2
180	7.449 ± 0.03	35.1 ± 1.6	95.3 ± 3.9	0.7 ± 1.4	23.8 ± 0.8	24.8 ± 0.8
240	7.449 ± 0.03	35.2 ± 2.1	103.0 ± 6.7	0.8 ± 1.7	23.1 ± 1.2	24.8 ± 1.1

APPENDIX B. Arterial blood gases and pH in animals receiving sodium nitrite (20 mg/kg iv) only. Data are presented as means with ± S.D.

Time in min	pH	pCO_2	pO_2	BE	HCO_{3^-}	CO_2ct
-10	7.376 ± 0.05	45.2 ± 3.5	80.8 ± 12.9	0.42 ± 3.4	25.9 ± 2.9	27.4 ± 2.9
-5	7.375 ± 0.06	45.4 ± 3.4	83.0 ± 12.9	0.38 ± 4.2	26.0 ± 3.6	27.5 ± 3.6
3	7.415 ± 0.06	38.8 ± 4.2	111.1 ± 28.7	-0.16 ± 3.2	24.2 ± 2.6	25.5 ± 2.5
5	7.410 ± 0.06	40.6 ± 2.6	94.2 ± 19.8	0.66 ± 4.5	25.3 ± 3.8	26.6 ± 3.8
10	7.413 ± 0.05	40.0 ± 2.5	79.4 ± 13.5	0.50 ± 3.8	25.0 ± 3.1	26.3 ± 3.2
15	7.417 ± 0.05	38.3 ± 4.9	77.5 ± 12.5	-0.18 ± 3.6	24.1 ± 3.3	25.3 ± 3.5
30	7.436 ± 0.03	41.6 ± 5.2	67.2 ± 18.3	2.52 ± 2.9	26.8 ± 3.2	28.2 ± 3.3
45	7.439 ± 0.03	40.4 ± 5.4	62.7 ± 6.8	2.68 ± 2.9	26.6 ± 3.0	28.0 ± 3.3
60	7.440 ± 0.03	39.9 ± 6.3	61.8 ± 6.4	2.44 ± 3.6	26.3 ± 4.0	27.6 ± 4.1
120	7.443 ± 0.04	40.2 ± 7.4	64.3 ± 5.3	2.92 ± 4.4	26.5 ± 4.7	27.8 ± 4.9
180	7.442 ± 0.04	40.2 ± 8.8	74.7 ± 3.9	3.0 ± 4.0	26.5 ± 4.8	27.7 ± 5.1
240	7.428 ± 0.04	41.8 ± 6.5	80.7 ± 6.9	2.96 ± 3.4	26.6 ± 3.6	27.8 ± 3.8

APPENDIX C. Arterial blood gases and pH in animals receiving sodium cyanide (8.4 mg/kg iv) and treatment with 4-DMAP (5 mg/kg im) at one minute. Data are presented as means with ± S.D.

Time in min	pH	pCO_2	pO_2	BE	HCO_3^-	CO_2ct
-10	7.415 ± 0.05	42.4 ± 6.3	103.8 ± 15.4	2.2 ± 3.8	26.2 ± 3.8	27.5 ± 3.9
-5	7.434 ± 0.08	43.4 ± 10.3	100.1 ± 6.1	4.1 ± 6.5	28.1 ± 6.3	29.4 ± 6.5
5	7.519 ± 0.08	30.7 ± 16.3	190.9 ± 38.8	-0.5 ± 3.5	21.1 ± 5.6	22.0 ± 6.1
10	7.539 ± 0.11	22.7 ± 14.4	105.7 ± 19.6	-2.1 ± 3.6	17.4 ± 5.2	18.1 ± 5.5
15	7.516 ± 0.08	21.3 ± 11.1	80.1 ± 17.1	-3.6 ± 4.9	16.3 ± 5.7	16.9 ± 5.9
30	7.474 ± 0.06	21.9 ± 6.9	67.1 ± 19.9	-4.7 ± 6.4	16.2 ± 6.0	16.8 ± 6.2
45	7.430 ± 0.07	23.9 ± 10.3	74.7 ± 20.9	-5.9 ± 7.2	15.9 ± 7.3	16.7 ± 7.6
60	7.404 ± 0.07	25.7 ± 11.1	79.3 ± 23.1	-6.3 ± 7.8	16.2 ± 7.8	17.1 ± 8.1
120	7.336 ± 0.14	33.6 ± 20.5	92.2 ± 15.2	-6.9 ± 8.9	17.6 ± 8.4	18.6 ± 8.9
180	7.477 ± 0.11	27.6 ± 6.7	110.0 ± 21.0	-1.8 ± 4.7	19.6 ± 3.2	20.5 ± 3.2

APPENDIX D. Arterial blood gases and pH in animals receiving sodium cyanide (8.4 mg/kg iv) and treatment with sodium nitrite (20 mg/kg iv) at 1-3 minutes. Data are presented as means with ± S.D.

Time in	pH	pCO_2	pO_2	BE	HCO_{3^-}	CO_2ct
-10	7.457 ± 0.04	41.4 ± 8.5	94.9 ± 13.6	4.6 ± 2.8	28.1 ± 3.8	29.4 ± 4.0
-5	7.461 ± 0.04	41.4 ± 8.7	95.4 ± 10.6	4.9 ± 2.7	28.3 ± 3.8	29.6 ± 4.0
5	7.527 ± 0.05	26.4 ± 3.1	175.0 ± 17.7	0.0 ± 3.7	20.9 ± 3.2	21.7 ± 3.3
10	7.437 ± 0.12	31.5 ± 9.4	125.6 ± 16.9	-2.6 ± 4.4	20.1 ± 3.6	21.1 ± 3.8
15	7.382 ± 0.17	32.5 ± 12.5	115.9 ± 15.7	-5.5 ± 6.1	18.1 ± 4.5	19.1 ± 4.7
30	7.392 ± 0.17	30.1 ± 14.4	87.6 ± 43.4	-5.7 ± 6.5	16.9 ± 4.9	18.0 ± 5.4
45	7.417 ± 0.15	29.7 ± 10.2	89.5 ± 20.8	-3.7 ± 10.2	19.2 ± 9.1	20.1 ± 9.3
60	7.469 ± 0.05	31.1 ± 10.9	94.8 ± 6.7	0.3 ± 9.1	22.4 ± 9.9	23.4 ± 10.2
120	7.406 ± 0.05	37.2 ± 6.5	90.1 ± 5.6	-0.8 ± 6.5	23.0 ± 6.5	23.0 ± 6.5
180	7.376 ± 0.17	44.2 ± 9.2	70.2 ± 41.8	0.9 ± 7.8	25.2 ± 6.3	26.5 ± 6.2

APPENDIX E. Arterial blood gases and pH in animals poisoned with sodium cyanide (8.4 mg/kg iv) and treatment with 4-DMAP (5 mg/kg im) at 1 minute and sodium thiosulfate (167 mg/kg iv) at 61-66 minutes.

Time in min	pH	pCO_2	pO_2	BE	HCO_3^-	CO_2ct
-10	7.432 ± 0.02	36.7 ± 8.3	95.9 ± 12.7	0.4 ± 3.7	23.6 ± 4.7	24.6 ± 4.8
-5	7.432 ± 0.02	40.2 ± 3.5	92.3 ± 8.7	2.5 ± 1.8	26.0 ± 2.1	27.2 ± 2.2
5	7.417 ± 0.15	37.3 ± 14.1	188.0 ± 50.7	-1.9 ± 2.4	21.7 ± 2.6	22.9 ± 2.9
10	7.446 ± 0.10	27.4 ± 7.4	122.1 ± 59.9	-4.1 ± 2.3	13.9 ± 1.7	18.7 ± 1.9
15	7.454 ± 0.09	23.1 ± 6.2	116.8 ± 9.3	-5.9 ± 2.1	15.4 ± 1.1	16.1 ± 1.2
30	7.475 ± 0.07	19.0 ± 1.8	76.4 ± 38.9	-6.8 ± 2.9	13.7 ± 1.7	14.3 ± 1.7
45	7.441 ± 0.08	22.0 ± 3.5	86.0 ± 13.0	-6.7 ± 4.2	14.8 ± 3.1	15.5 ± 3.1
60	7.421 ± 0.08	24.1 ± 3.9	79.9 ± 9.1	-6.6 ± 4.6	15.5 ± 3.6	16.2 ± 3.6
70	7.348 ± 0.08	28.4 ± 4.7	68.7 ± 10.7	-7.6 ± 3.7	16.2 ± 3.7	17.2 ± 3.9
80	7.361 ± 0.03	32.6 ± 5.5	73.9 ± 11.1	-5.9 ± 3.3	18.0 ± 3.4	18.9 ± 3.5
90	7.371 ± 0.03	34.1 ± 5.1	75.0 ± 13.8	-4.6 ± 2.9	19.5 ± 2.9	20.3 ± 3.2
120	7.404 ± 0.03	36.6 ± 3.4	76.5 ± 15.9	-1.4 ± 0.9	22.2 ± 1.1	23.3 ± 1.2
180	7.433 ± 0.03	36.9 ± 5.3	86.1 ± 7.7	0.7 ± 1.4	23.8 ± 2.2	24.9 ± 2.3

APPENDIX F. Arterial blood gases and pH in animals poisoned with sodium cyanide (8.4 mg/kg iv) and treatment with sodium nitrite (20 mg/kg iv) at 1-3 minutes and sodium thiosulfate (167 mg/kg iv) at 61-66 minutes. Data are presented as means ± S.D.

Time in min	pH	pCO_2	pO_2	BE	HCO_3^-	CO_2ct
-10	7.416 ± 0.04	39.5 ± 5.5	98.1 ± 6.1	0.7 ± 2.8	24.6 ± 2.9	25.8 ± 3.0
-5	7.427 ± 0.04	39.4 ± 5.5	100.9 ± 6.7	1.4 ± 2.1	25.0 ± 2.4	26.2 ± 2.6
5	7.446 ± 0.09	31.9 ± 14.6	171.4 ± 21.6	-2.3 ± 2.8	20.1 ± 4.8	21.1 ± 5.2
10	7.523 ± 0.06	21.6 ± 7.2	138.1 ± 8.0	-3.4 ± 3.2	16.8 ± 4.0	17.5 ± 4.2
15	7.452 ± 0.06	24.2 ± 8.2	129.9 ± 9.6	-5.6 ± 3.3	16.1 ± 4.1	16.8 ± 4.3
30	7.512 ± 0.09	18.2 ± 2.0	112.3 ± 13.1	-5.6 ± 3.7	14.3 ± 2.4	14.9 ± 2.3
45	7.475 ± 0.06	22.1 ± 1.1	97.7 ± 10.6	-5.0 ± 3.8	16.1 ± 2.9	16.7 ± 2.9
60	7.442 ± 0.05	25.2 ± 2.0	87.0 ± 9.6	-4.9 ± 3.3	16.9 ± 2.8	17.8 ± 2.8
70	7.379 ± 0.02	30.5 ± 3.5	77.5 ± 10.9	-5.9 ± 2.7	17.5 ± 2.6	18.4 ± 2.8
80	7.387 ± 0.03	30.7 ± 4.5	77.1 ± 7.9	-5.8 ± 3.5	17.9 ± 3.3	16.9 ± 7.8
90	7.398 ± 0.03	31.6 ± 4.9	75.3 ± 10.9	-4.3 ± 3.2	18.9 ± 3.3	19.9 ± 3.5
120	7.414 ± 0.03	32.2 ± 4.8	77.2 ± 9.3	-2.3 ± 4.2	20.1 ± 3.9	21.2 ± 3.9
180	7.438 ± 0.03	33.9 ± 4.1	76.4 ± 6.4	-0.50 ± 3.5	22.3 ± 3.6	23.4 ± 3.7

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